Microfabricated Polymeric Vessel Mimetics for 3D Cancer Cell Culture in Matrigel

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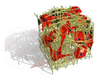
INTRODUCTION

Delivering a new drug to market in the United States costs an average of $1 billion dollars over 15 years[1]. The failure rate of drugs entering early clinical trials is about 85%[2]. The divergence between results from preclinical studies and efficacy in human clinical trials is a persistent challenge in drug discovery.

Animal models are expensive, time consuming, and inadequate in predicting clinical efficacy

2D monolayer cell culture fails to recapitulate the biological cues and cell-cell interaction found in the *in vivo* tumor microenvironment



Standard 3D scaffolds and matrices lack vasculature, thus limiting the transport of O_2 and other nutrients necessary for sustaining metabolism and growth.

Here we report a 3D cell culture system using a microfabricated polymeric vessel mimetic that facilitates O_2 delivery to cancer cell cultures in anoxia. This study has implications for the development of an *in vitro* tumor models that mimic the tumor microenvironment.

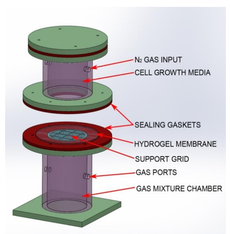
OBJECTIVES

Aim 1: Bioreactor design. Use microfabricated polydimethylsiloxane (PDMS) pillars to "vascularize" 3D cell cultures and mimic *in vivo* oxygen perfusion

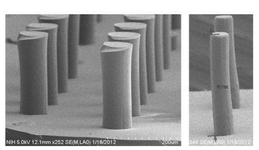
Aim 2: Validate cell culture growth pattern. Evaluate the effect of the oxygen gradient on the growth patterns of tumor cell cultures

Aim 3: Culture cells for drug screening. Develop and optimize techniques to use high-throughput, quantitative cytotoxicity assays for 3D cultures.

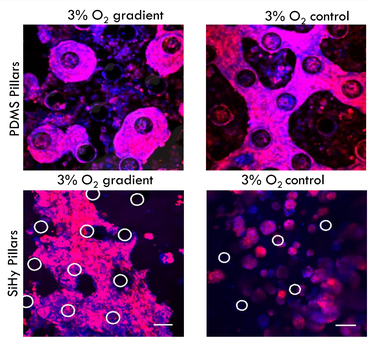
1st GENERATION BIOREACTOR



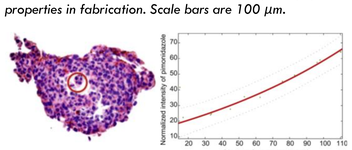
Single chamber bioreactor system (11.5 cm x13 cm): two acrylic chambers separated by membrane with pillars (either PDMS or silicone hydrogel). Continuous gas flow maintains each chamber at its desired oxygen concentration[3].



Micropillars for oxygen delivery are fabricated using template of SU-8 micropillars with 100 µm diameter and 250 µm height. Using the SU-8 master, negative PDMS molds were replicated, and silicone hydrogel micropillars were cast from PDMS molds. Dimensions and spacing of pillars are designed to mimic those of capillaries *in vivo*[3].

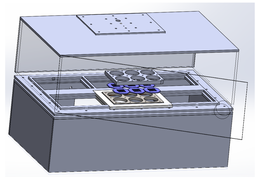


OVCAR8-dsRed2 cells with Hoechst stain after 7 d culture on PDMS (top) and silicone hydrogel (bottom) pillars. PDMS replaced SiHy as the pillar material in later design due to improved batch-to-batch consistency of physical properties in fabrication. Scale bars are 100 µm.

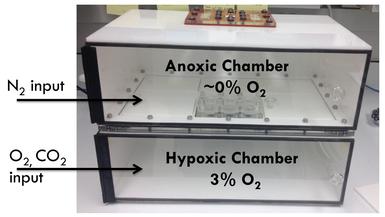


OVCAR8-dsRed2 cells grown for 7 days on silicone hydrogel pillars with a 3% O_2 gradient were stained with hypoxia-marking dye pimonidazole and imaged (*left*). Cells become hypoxic about 100 µm from the pillar (circled in red), comparable to *in vivo* results[3].

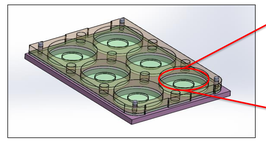
2nd GENERATION BIOREACTOR



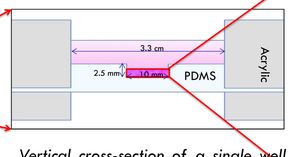
The bioreactor is designed using SolidWorks and fabricated by laser cutting acrylic sheets. The top chamber and bottom chamber were sealed by gas-tight gaskets.



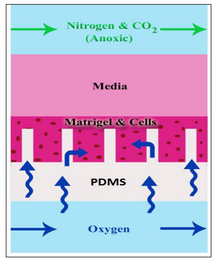
The redesigned bioreactor chamber accommodates 6 custom six-well cell culture plates for high-throughput experiments. The system features feedback control for the O_2 concentration in the hypoxic chamber and real-time monitoring of O_2 levels.



Custom six-well plate assembly: two acrylic plates in the six-well plate format secure the PDMS membrane in the middle using rare-earth magnets.



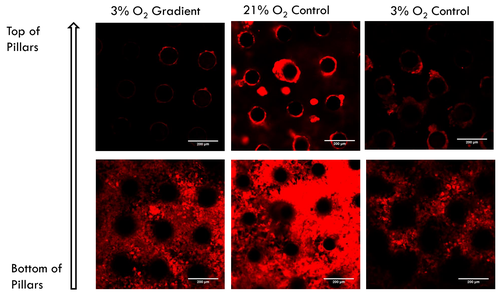
Vertical cross-section of a single well on the PDMS membrane. Each well contains an inset miniwell with microfabricated micropillars lining the bottom, where cells in Matrigel are seeded.



Vertical cross-section of a single miniwell containing micropillars. PDMS replaced silicone hydrogel as the material for the pillars because of increased batch-to-batch consistency in fabrication. Cells are embedded in Matrigel and seeded over the pillars, which deliver oxygen to the 3D cell layer.

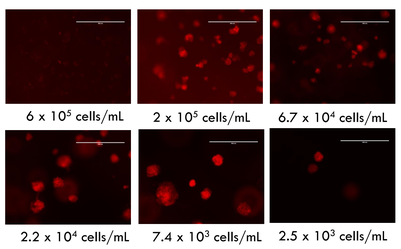
3D CELL CULTURE IN BIOREACTOR

Troubleshooting Spheroid Growth Patterns



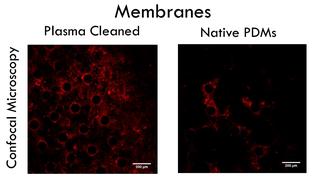
OvCar-dsRed2 cells were cultured for 7 days in Matrigel on plasma cleaned PDMS membranes with pillars in a 3% O_2 gradient, as well as no-gradient control environments. Preliminary results show that cells preferentially attached to the bottom of the membrane instead of forming spheroids in the Matrigel. Cell layers can be seen around the pillars regardless of the oxygen gradient. Scale bars are 200 µm.

Cell Density Optimization



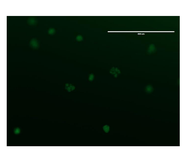
To optimize baseline culture conditions, OvCar-dsRed2 cells were seeded in 2.5 mg/mL BD Matrigel at concentrations from $6x10^5$ to 2.5×10^3 and monitored for 7 days in a 96-well plate at 21% oxygen. The optimal spheroid density and cluster size is observed for concentrations between 2.2×10^4 cells/mL and 7.4×10^3 cells/mL. Scale bars are 400 µm.

The Effect of Plasma Cleaning PDMS Membranes



While plasma cleaning renders native PDMS hydrophilic and improved the homogenous deposition of Matrigel on the PDMS membrane, plasma cleaning also caused preferential cell adhesion on PDMS surfaces on the bottom of the membrane and on the pillars, thus confounding results. Plasma cleaning was therefore discontinued. Scale bars are 200 µm.

Fluorescent Tracking of Cancer Cell Lines

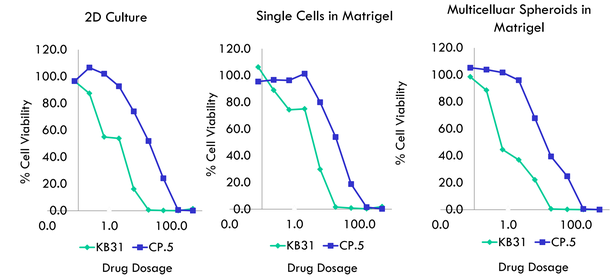


CellTracker (Life Technologies), a fluorescent dye, was adapted for cell cultures in Matrigel and was used to visualize OvCar8 spheroids for live cell tracking. This technique can be used to visualize other non-fluorescent cell lines for culture in the bioreactor. Scale bar is 400 µm.

Confocal Imaging of Custom Plates for Longitudinal Studies

A custom stage adaptor was designed in SolidWorks and 3D printed, to allow for confocal imaging of the intact assembly using a standard microscope. Keeping the stage intact enables longitudinal monitoring, instead of endpoint imaging only.

CYTOTOXICITY ASSAY

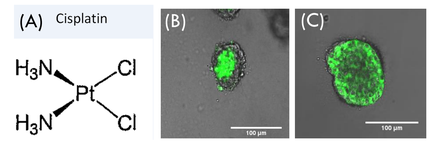


	KB 3-1	CP.5
IC_{50} (µM)	2.480	21.031
SD (µM)	0.102	2.831

	KB 3-1	CP.5
IC_{50} (µM)	4.471	22.921
SD (µM)	0.702	4.633

	KB 3-1	CP.5
IC_{50} (µM)	9.356	32.133
SD (µM)	0.702	6.868

A protocol was developed for performing cytotoxicity assays in cells cultured in Matrigel using CellTiter-Glo®. The assay shows that, as expected, cell resistance is escalated for multicellular spheroids as compared to single cells. Dose response curves of Kb3-1 cells, a parental cell lines, and Cp.5, a cisplatin-resistant cell line, are shown above for cisplatin, a platinum based anti-cancer drugs.



(A) The chemical structure of Cisplatin, a platinum based anticancer drug. (B) KB 3-1 spheroids uptake cisplatin conjugated with BODIPY dye in the center of the spheroid and (C) uptake free dye in cells throughout the spheroid.

FUTURE WORK

I. Further characterization of the bioreactor system

- Growth pattern and cell localization
- RNA expression
- Drug sensitivity/ penetration
- Hypoxia markers

II. Culture other cancer cell lines in bioreactor

- Breast – MCF7
- Ovarian- OvCar 8
- Kidney-SN12C
- AML - CEM
- Liver- FOCUS
- Colon- DLD1

ACKNOWLEDGMENTS

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